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## Combined electrochemistry and Raman spectroscopy of heme proteins

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## Abstract English

Protein investigation is attracting nowadays substantial research efforts because of the fundamental interests and the potential practical applications in many different fields, such as the development of bioelectronic devices, pharmacology, medicine and environmental sciences. Intriguing applications that ten years ago were only predicted at a theoretical level – mimicking neuronal coupling of neurons with electronic devices (“brain computers”), designing integrated sensors that might detect high glucose levels and release insulin, and engineering biofuel cells that might use body fluids as fuel for generating electricity – are nowadays experimentally explored. This challenge has stimulated scientists with different expertise to cooperate, giving rise to a multidisciplinary approach, which is probably the most remarkable feature related to protein studies.

This thesis is meant to be a small but significant contribution to this interdisciplinary approach, since it is aimed at linking two important analytical techniques in protein investigations, i.e. electrochemistry and Raman spectroscopy. This approach – consisting in performing experiments with both techniques simultaneously on the same electrode sample – aims at achieving information about the relation between structure and functionality of proteins, as well as a deeper understanding of charge transport phenomena through biological matrices.

**Chapter 1** contains a brief introduction about cytochrome *c*, (cyt *c*) – the heme protein chosen as sample to test our spectroelectrochemical setup – as well as some basic concepts about the two techniques used in this thesis and their relevance in protein study. The description of the theoretical basis of cyclic voltammetry (CV) and Raman spectroscopy are kept at a very simple level. This was done to introduce the advantages of our experimental strategy to scientist who might be already involved in protein studies, but are focusing on different analytical techniques. Chapter 1 was thought to offer them a guideline to better understand the applicability of combined CV and SERRS to their field of interest. However, for those who are interested, the following overviews of CV and Raman spectroscopy are recommended: Broadening Electrochemical Horizons (Bond, A.

M. 2002, Oxford Science Publications), and *Vibrational Spectroscopy in Life Science* (Siebert F.; Hildebrandt, P. 2007 Wiley).

**Chapter 2** describes the making of the spectroelectrochemical cell required to perform combined CV and Raman spectroscopy of heme protein. This small tool has been modified during the years according to our needs, and this chapter includes the original cell design (published material) with a description of the subsequent modifications (unpublished material).

**Chapter 3** shows how the combination of CV and Raman spectroscopy has been used to investigate the properties of cyt *c* immobilized at different electrodes.

One of the main reasons this combination of CV and Raman spectroscopy was particularly challenging is the metal substrate needed for SERRS measurements (i. e. roughened silver), because protein electrochemistry prefers usually different electrode materials. In **Chapter 4** we investigated systematically the differences between the redox behavior of cyt *c* at gold and silver surfaces, in order to establish if the metal of choice did influence the redox behavior of the protein.

Even if our interests were focused mainly on cyt *c*, other heme proteins were also tested with the spectroelectrochemical approach. **Chapter 5** reports two examples of heme proteins (cyt *c'* and cytochrome P450), including the strategy for preparing and characterizing a hybrid bilayer membrane (HMB) at SERS-active silver electrodes. This artificial membrane supported by a solid electrode is a relative recent development and its potentiality in tailoring membrane proteins are not fully explored yet. In this scenario, the engineering of HBM at SERS-active electrodes is extremely interesting in view of possible future research.

In **Chapter 6** some conclusions on the state of the art about combined CV and SERRS are briefly outlined, including a frequently asked questions section which is aimed to stimulate the debate about the topics discussed in this thesis. Possible guidelines for future developments in this field are also discussed.